

UNCLASSIFIED

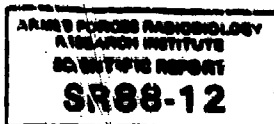
DTIC FILE COPY

(P)

AD-A200 642

ORT DOCUMENTATION PAGE

1b. RESTRICTIVE MARKINGS	
3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) AFRRI SR88-12	
5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION Armed Forces Radiobiology Research Institute	6b. OFFICE SYMBOL (If applicable) AFRRI
7a. NAME OF MONITORING ORGANIZATION	
7b. ADDRESS (City, State and ZIP Code) Defence Nuclear Agency Bethesda, Maryland 20814-5145	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Defense Nuclear Agency	8b. OFFICE SYMBOL (If applicable) DNA
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
10. SOURCE OF FUNDING NOS.	
PROGRAM ELEMENT NO. NWED QAXM	PROJECT NO.
TASK NO.	WORK UNIT NO.
11. TITLE (Include Security Classification) (see below)	
12. PERSONAL AUTHOR(S) Joseph, J.A., Dalton, T.K., and Hunt, W.A.	
13a. TYPE OF REPORT Scientific Report	13b. TIME COVERED FROM TO
14. DATE OF REPORT (Yr., Mo., Day) Apr-May-June 1988	
15. PAGE COUNT 9	
16. SUPPLEMENTARY NOTATION Published in Brain Research, 454 (1988)	
17. COSATI CODES	
FIELD	GROUP
SUB. GR.	
18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Title: Age-related decrements in the muscarinic enhancement of K ⁺ -evoked release of endogenous striatal dopamine: an indicator of altered cholinergic-dopaminergic reciprocal inhibitory control in senescence.	
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS <input type="checkbox"/>	
21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL Junith A. Van Deusen	22b. TELEPHONE NUMBER (Include Area Code) (202) 295-3536
22c. OFFICE SYMBOL ISDP	



Age-related decrements in the muscarinic enhancement of K^+ -evoked release of endogenous striatal dopamine: an indicator of altered cholinergic--dopaminergic reciprocal inhibitory control in senescence

J.A. Joseph, T.K. Dalton and W.A. Hunt

Department of Behavioral Sciences, The Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145 (U.S.A.)

(Accepted 19 January 1988)

Key words: Striatum; Dopamine autoreceptor; Muscarinic; Aging

Previous experiments have indicated that the release of striatal dopamine (DA) is controlled by inhibitory DA autoreceptors which are mediated by inhibitory cholinergic heteroreceptors (HTRs). Activation of the HTRs by muscarinic or nicotinic agonists potentiates the K^+ -evoked release of DA from the striatum. Present experiments were carried out to determine if this relationship is altered as a function of aging. Cross-cut striatal tissue slices obtained from 3 age-groups (6, 12-18 and 24 months) Wistar rats were superfused with a modified Krebs-Ringer basal release medium containing 2.5 mM KCl. After a 30-min equilibration period, a 5-min baseline fraction was collected from each chamber. The medium was then switched to one containing 30 mM KCl, and depending upon the experiment, 1 of 4 concentrations of a particular muscarinic (oxotremorine, pilocarpine, carbachol or bethanecol) or nicotinic (nicotine) agonist. In some experiments DA autoreceptor function was assessed directly with haloperidol. Six 5-min fractions were taken during depolarization. DA release was assessed using high performance liquid chromatography coupled to electrochemical detection. Results indicated that the efficacy of the muscarinic agonists was reduced in an age-dependent manner with the oldest age groups showing the smallest enhancement. The age at which the decline was seen was dependent on the muscarinic agonist that was applied. Deficits were seen as early as 12 months when full agonists (e.g. carbachol) were applied, but did not appear until 18 months when partial agonists (e.g. oxotremorine) were applied. These age-related alterations were not seen when haloperidol or nicotine were used to enhance the K^+ -evoked release of DA. Results are discussed in terms of specific deficits in the muscarinic control of striatal DA autoreceptors in senescence. These decrements occur as result of age-related alterations in the conformational/orientational state of the muscarinic receptors regulating striatal DA release.

INTRODUCTION

Evidence derived from numerous sources indicates that there are very significant changes occurring within both the striatal dopamine (DA) and acetylcholine (ACh) systems during aging. The exact specification of these alterations is dependent upon both the species examined and the conditions of the experiment, but indications are that some of the most striking changes take place in the various populations of receptors from these systems. In the case of the striatal DA system, the receptors were originally divided into two subtypes (D_1 and D_2 ref. 17), and subsequent findings indicated that each receptor subtype

showed a different pattern of change with age. It appeared that while the D_2 -receptor showed remarkably consistent age-related declines in concentration, the changes occurring in the concentration of striatal D_1 -receptors was much more variable. Initial assessments of D_2 -receptor loss with age indicated a 35% decrease in binding with no loss of binding affinity in the aged rat. Subsequent observations have indicated that the degree of D_2 -receptor loss is dependent upon the assay conditions, the strain or species of animal tested, and the particular dopamine-sensitive ligand that is utilized, but this decline generally ranges from about 36 to 66%^{11,15,31,32}.

If age-related changes in the B_{max} or K_d of the stri-

Correspondence: J.A. Joseph, Department of Behavioral Science, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145, U.S.A.

88 1012 087

tal D_1 -receptor are considered, the results from at least one laboratory show parallel losses between D_1 -receptor concentration and DA-stimulated adenylate cyclase activity that are the greatest between 3 and 12 months of age in the rat¹¹. However, findings from laboratories using other strains of rats (e.g. Sprague-Dawley²⁴) or mice (e.g. C57BL6 ref. 24) indicate no change in the concentration of striatal D_1 -receptors with age. Examination of postmortem human material indicates an actual increase in D_1 -receptors with age²². Since the changes in the concentration of striatal D_2 -receptors seem to follow a progressive decline throughout the life-span of the organism, while changes in the concentration of striatal D_1 -receptors do not, these results suggest that there may be increases in the striatal D_1/D_2 -receptor ratio in senescence, a finding which has been confirmed in humans²² and rats¹¹.

Assessments of age-related differences in striatal muscarinic cholinergic receptor binding indicate that there are decreases in the number of these binding sites in the striatum as a function of age. Declines in the concentration of striatal muscarinic receptors of about 25–29% in the rodent have been reported in studies using the ligand, [3H](–)-quinuclidinyl benzilate ([3H]QNB²³). These declines were seen primarily in the medial and caudal portions of the striatum³⁴, a pattern that approximates that seen with respect to loss of D_2 -receptors.

One question that arises from these findings is that since there appears to be a great deal of interactive modulation that takes place between the striatal DA and ACh systems, how do age-related declines in their concentrations alter these respective interactions? This question has not received much systematic study, but there are indications that there may be age-related declines in the normal reciprocal inhibitory control (RIC) that is exerted between these two systems. Recent studies have shown that while striatal DA-stimulated adenylate cyclase activity is inhibited by muscarinic agonists such as oxotremorine or carbachol in striatal broken cell preparations prepared from young rats (6 months), these agents were ineffective in inhibiting this activity in senescent rats (24 months)⁴.

Additional assessments carried out to examine DA modulation of [3H]ACh release from striatal slices from mature, middle-aged and senescent rats indi-

cated that the DA agonist, apomorphine, was effective in inhibiting K^+ -evoked release of [3H]ACh from superfused striatal slices obtained from mature and middle-aged rats but not from those prepared from old rats³⁵.

These findings, when considered in light of those discussed above indicate that as receptors from both of these transmitter systems are lost with age, alterations in one may profoundly affect the functioning of the other, and RIC that is normally exerted between these two systems is significantly diminished. The present experiments were directed toward further examination of age-related alterations in cholinergic regulation of striatal DA autoreceptors. It is now widely accepted that striatal DA release is under the control of a group of inhibitory DA autoreceptors^{1–7,12,13,16,18,25,26,33,40}. If these autoreceptors are stimulated with DA agonists or inhibited with DA antagonists, K^+ -evoked release of DA will be respectively inhibited or enhanced. Normally, this control is in turn mediated through inhibitory muscarinic and/or nicotinic heteroreceptors^{19,27,28,30,41} presumably located on the same terminals as the autoreceptors¹⁰. Either muscarinic (e.g. oxotremorine, carbachol, bethanecol) or nicotinic (e.g. nicotine) agonists can activate the heteroreceptors, which inhibit the DA autoreceptor and potentiate the K^+ -evoked release of DA from the striatum^{27,28}. The actions of the muscarinic agonists on DA release can be attenuated by both atropine as well as the more selective M_1 -muscarinic antagonist pirenzepine²⁶.

Thus, enhancement of DA release was assessed in superfused striatal slices obtained from young, middle-aged, and old rats following application of muscarinic or nicotinic agonists and muscarinic antagonists. Additionally, in order to examine any age-related alterations in DA autoreceptor function directly, the potentiation of K^+ -evoked DA release by haloperidol was also determined.

MATERIALS AND METHODS

Procedure

Striatal tissue from mature (6 months), middle-aged (12 and 18 months), and aged (24 months) Wistar rats was obtained (following sacrifice by decapitation) from the Gerontology Research Center, Baltimore, MD (in some experiments striata from only 6-



A-1 20

and 24-month rats were utilized; see below). Cross-cut striatal slices (0.3 μm) were prepared using a McIlwain tissue chopper. Slices from equal numbers of animals were pooled for each age group and placed in small glass vials containing a modified Krebs-Ringer basal release medium that had been bubbled for 30 min with 95% O_2 /5% CO_2 and contained (in mM) NaHCO_3 21, glucose 3.4, NaH_2PO_4 1.3, EGTA 1, MgCl_2 0.93, NaCl 127 and KCl 2.5 (pH 7.4). The slices were washed twice in this medium and 150 μl aliquots (approximately 1.1–1.4 mg tissue/chamber) were placed in a superfusion apparatus containing 16 parallel chambers. Typically, pooled tissue from 2 animals was used to fill 4 of the parallel chambers. The tissue and buffer media were maintained at 37 $^\circ\text{C}$ throughout the course of the experiment. Following placement into the superfusion chambers, the tissue was allowed to equilibrate for 30 min. It was continuously perfused with the oxygenated basal release medium at the rate of 124 $\mu\text{l}/\text{min}$. After the equilibration period, a 5-min baseline fraction was collected and the medium was then switched to one containing (in mM) KCl 30, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1.26 (in place of EGTA) and NaCl 57, as well as the other components described above (pH 7.4). Following the switch, 5-min fractions continued to be collected for 30 min. To this oxygenated release medium was added 1 of 4 concentrations of the agent under study ([0 μM , 100 μM , 500 μM , and 1 mM of oxotremorine, carbachol, pilocarpine, bethanecol, or nicotine]; (haloperidol, 0 μM , 10 μM , 100 μM , 500 μM)). In addition, the specificity of any muscarinic agonist enhancement of KCl-induced release of DA was examined following application of 500 μM oxotremorine in the presence or absence of graded concentrations of atropine (100 μM , 500 μM , 1000 μM) or the selective M_1 antagonist pirenzepine (500 μM , 1000 μM , 2000 μM). Although only one agonist or antagonist was studied in a particular experiment, all concentrations were represented in that experiment. The fractions were collected into tubes on ice containing 0.3 ml of cold 0.1 N perchloric acid, 0.05% sodium metabisulfite, and 0.1% EDTA. These samples were then stored at -80°C until assessment of DA release via high performance liquid chromatography (HPLC) coupled to electrochemical detection.

The HPLC consisted of a varian model 5000 ternary chromatograph, a varian 401 data system, a var-

ian model 8055 autosampler, and a valco air actuated injector with a 50 μl loop. The effluent was monitored with a bioanalytical systems LC-4B amperometric detector using a glassy carbon electrode. The detector potential was set at 0.72 V as a Ag/AgCl_2 reference electrode with a sensitivity of 10 nA/V. The mobile phase consisted of a filtered, degassed 100 mM KH_2PO_4 buffer containing 3 mM 1-heptanesulfonic acid, 100 μM EDTA, and 8% (V/V) acetonitrile (pH 3.6). The components were eluted off a Waters 10 μm particle, $\mu\text{BondapakC}^{18}$ reverse-phase column (30 \times 0.39 cm; flow rate = 1 ml/min) maintained at 30 $^\circ\text{C}$. Results were calculated relative to

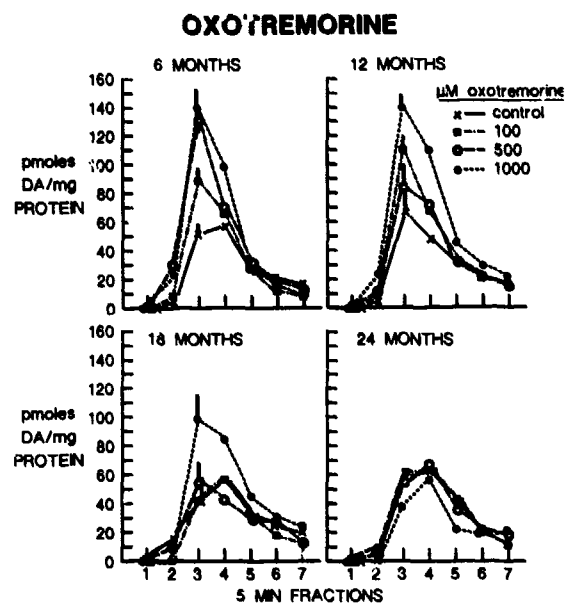


Fig. 1. Release of dopamine (DA) from superfused striatal tissue slices following stimulation by 30 mM KCl and 1 of 4 concentrations of oxotremorine. Striata were obtained from Wistar rats of 4 age groups. The slices were placed in a superfusion apparatus containing 16 chambers. Pooled tissue from two animals was used to fill 4 chambers. The slices were allowed to equilibrate for 30 min and were continuously perfused with oxygenated basal release medium (see Methods) at the rate of 124 $\mu\text{l}/\text{min}$. After the equilibration period a 5 min baseline release fraction was collected. The medium was then switched to one containing the 30 mM KCl and one of the compounds under study. Six additional 5-min fractions were then taken. Dopamine release was analyzed by high performance liquid chromatography coupled to electrochemical detection. Note that the peak release of DA was not seen until the second fraction after the switch (3 on the x-axis). This delay is the result of the time required in getting the high KCl medium from the reservoir to the tissue and then to the collection vial. The size (PE50), and length (112 cm) of the tubing as well as the flow rate dictated this delay to be about 5–7 min after the switch.

known standards that were analyzed on the HPLC at the same time as the samples. Samples were run in an autosampler and standards were placed at the beginning, middle and end of the sequence. Concentrations of the components were determined from areas under each peak when compared to the conversion factors derived from the standards. The lower limit of the sensitivity of this assay was 250 fmol. In order to ensure that DA was being assessed retention times were measured using DA standards. Additionally, pilot experiments were carried out to determine if DA 'spikes' added to samples enhanced the DA peaks on the chromatograms. Data were expressed as pm/mg protein as analyzed by the Lowry²¹ procedure.

Data analysis

In experiments where 4 age groups were represented (i.e. those which examined enhanced release of striatal dopamine induced by 30 mM KCl co-applied with oxotremorine, carbachol, pilocarpine, or bethanecol) data were analyzed using 4 (age) by 4 (drug concentrations) by 7 (fractions) repeated measures (on the last factor) analyses of variance. In experiments where nicotine, haloperidol, and atropine were examined, striata from the two groups of middle aged animals were not studied. Therefore, 2 (age) by 4 (drug concentrations) by 7 (fractions) analyses of variance were carried out. Further comparisons among the various groups were analyzed by post hoc *t*-tests⁴².

RESULTS

Muscarinic agonists and the enhancement of K⁺-evoked release of dopamine

Generally, as can be seen from Figs. 1-4, the release of DA in striatal slices obtained from all of the age groups was increased over basal release when a depolarizing concentration of KCl (30 mM) was introduced into the superfusion medium. These figures also show that in the striata obtained from the 6-month animals there was a concentration-dependent enhancement of the K⁺-evoked release of DA by oxotremorine (drug concentration by fraction — $F_{18,546} = 9.34$, $P < 0.0001$, Fig. 1; pilocarpine $F_{18,366} = 8.14$, $P < 0.0001$, Fig. 2; carbachol, $F_{18,234} = 1.97$,

$P < 0.01$, Fig. 3; and bethanecol $F_{18,402} = 3.27$, $P < 0.01$, Fig. 4). However, Figs. 1-4 also show that there were significant declines in the ability of these agonists to enhance K⁺-evoked release of DA as a function of age (drug concentration by fraction by age — oxotremorine, $F_{54,546} = 1.72$, $P < 0.001$; pilocarpine, $F_{54,366} = 1.62$, $P < 0.001$; bethanecol, $F_{54,402} = 3.83$, $P < 0.0001$; carbachol, $F_{54,234} = 1.71$, $P < 0.001$).

The pattern of these age-related declines was highly dependent upon the particular agonist that was examined. Enhancement of K⁺-evoked release of DA was generally higher in striata obtained from the 6-month groups than those from the 18- and 24-month groups when either oxotremorine or pilocarpine (from 100 μ M to 1000 μ M) was co-applied with 30 mM KCl (post hoc *t*-analyses, all comparisons of either 6-month groups with those of 18- or 24-months, $P < 0.05$). Similarly, significant differences in enhancement of K⁺-evoked release by oxotremorine were also observed between the 12-month groups and the older age groups (e.g. oxotremorine, 100 μ M, 12 vs 18 months — $t = 5.62$, $P < 0.001$; 500 μ M, $t = 3.07$, $P < 0.01$; 1000 μ M, $t = 4.35$, $P < 0.05$, $df = 546$; pilocarpine 1000 μ M, $t = 1.96$, $P < 0.05$, df

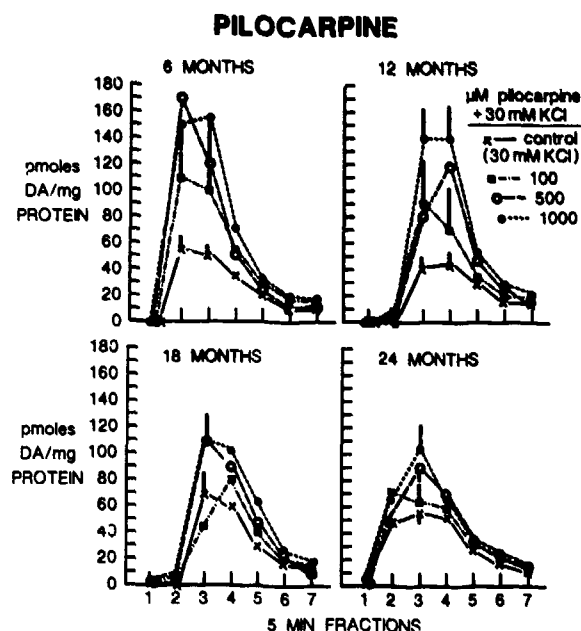


Fig. 2. Effects of pilocarpine on the K⁺-evoked release of dopamine from superfused striatal tissue slices obtained from different aged rats (6, 12, 18, and 24 months).

= 366). There was also some evidence of continued further decline in this aspect of RIC between 18 and 24 months, since subsequent post hoc *t*-analyses indicated significant differences between these two groups at the higher concentrations of both pilocarpine and oxotremorine (e.g. oxotremorine, 1000 μ M, $t = 4.35$, $P < 0.001$, $df = 546$; pilocarpine, 500 μ M, $t = 3.10$, $P < 0.02$, $df = 366$).

In contrast to the findings seen with respect to oxotremorine and pilocarpine, when carbachol was used to enhance K^+ -evoked release of DA there were indications that significant decrements in this enhancement appeared as early as 12 months of age (Fig. 3). Subsequent analyses indicated that there was less enhancement of K^+ -evoked release of DA in the striata from the 12-month group than those from the 6-month group for all concentrations of carbachol (i.e. 100 μ M, $t = 2.11$, $P < 0.02$; 500 μ M, $t = 5.15$, $P < 0.001$; 1000 μ M, $t = 5.55$, $P < 0.001$, $df = 254$). In fact, the only enhancement of K^+ -evoked release of DA that was observed in any of the striatal slices was seen in the 6-month group. Carbachol did not enhance DA release from the striata of any of the other groups above that seen with the application of KCl alone (all

within-age-group *t*-tests between 0 μ M and other concentrations of carbachol in the 12-, 18-, and 24-month age-groups $P > 0.05$).

When bethanecol was used to enhance the K^+ -evoked release of DA from striatal slices it can be seen from Fig. 4 that the results were similar to those seen with respect to carbachol in that the age-related deficits in the efficacy of bethanecol appeared in striata from the 12-month group (6 vs 12 months — 100 μ M, $t = 3.05$, $P < 0.02$; 500 μ M, $t = 4.72$, $P < 0.001$; 1000 μ M, $t = 5.96$, $P < 0.001$, $df = 400$) and continued into the 18- and 24-month groups (e.g. 6 vs 18 months — 500 μ M, $t = 4.55$, $P < 0.001$; 6 vs 24 months — 500 μ M, $t = 5.29$, $P < 0.001$, $df = 400$). Enhancement of the release DA by the various concentrations of bethanecol in the striata from the 12 month group did not differ from that seen in the striata from the 18 month group. Response differences between the striata from the 12- and 24-month groups were only seen when 500 μ M bethanecol was co-applied with 30 mM KCl ($t = 2.55$, $P < 0.01$, $df = 400$). These findings indicate that the age-related pattern of declines in responses tended to be similar between oxotremorine and pilocarpine wherein they were seen to begin in the 18- to 24-month groups.

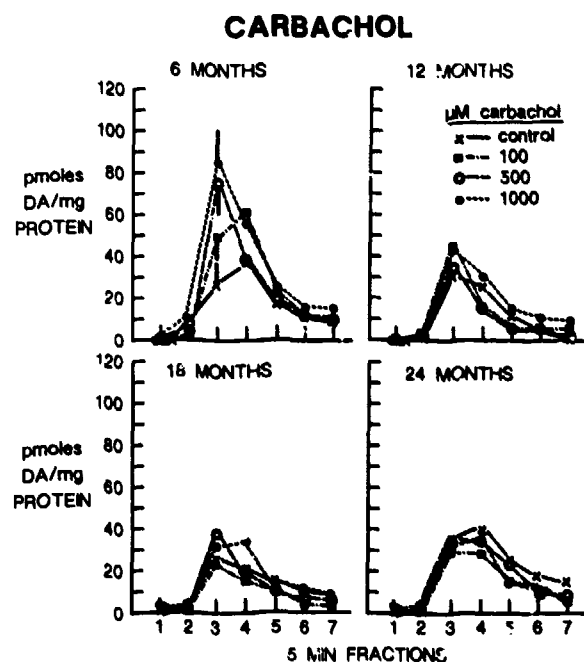


Fig. 3. Effects of carbachol on the K^+ -evoked release of dopamine from superfused striatal tissue slices obtained from rats of 4 different ages (6 through 24 months).

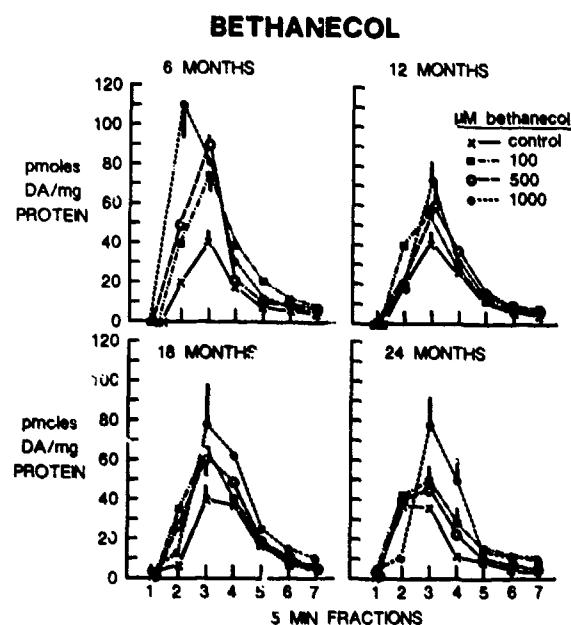


Fig. 4. Release of dopamine from superfused striatal tissue slices following stimulation by 30 mM KCl and 1 of 4 concentrations of bethanecol.

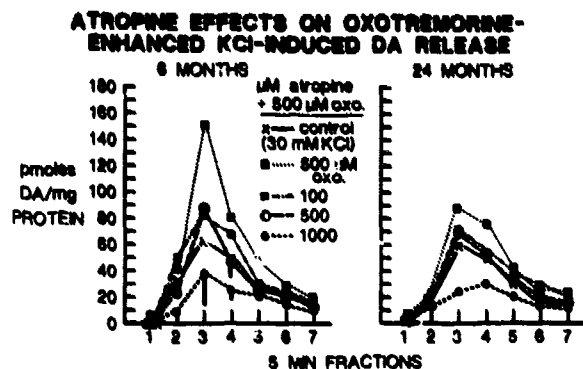


Fig. 5. Atropine antagonism of oxotremorine-enhanced, K^+ -evoked release of dopamine from superfused striatal tissue slices (6- and 24-month animals).

while the findings with respect to carbachol and bethanecol indicated that the deficits appeared as early as 12 months of age.

Importantly, as Fig. 5 indicates, there was a concentration-dependent antagonistic effect of atropine on oxotremorine-enhanced K^+ -induced release of striatal DA (concentration by fraction — $F_{24,126} = 4.03$, $P < 0.0001$). Similar findings were seen with respect to pirenzepine (concentration by fraction — $F_{24,120} = 4.44$, $P < 0.0001$) (Data not shown).

Nicotine enhancement of K^+ -evoked release of DA

Fig. 6 shows that unlike the muscarinic agonists, nicotine was much more effective in enhancing KCl-induced release of DA in the striata from old animals. While analysis of variance indicated that age-related differences were still present between the two

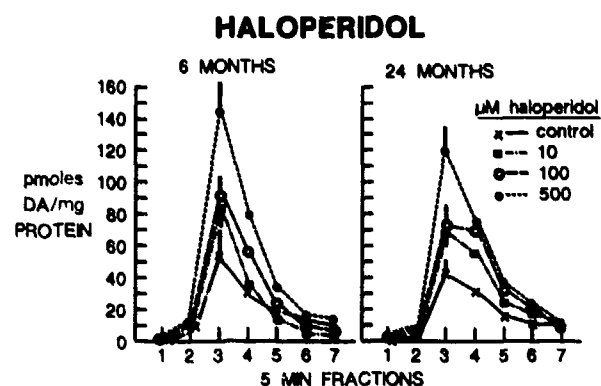


Fig. 6. Haloperidol enhancement of 30 mM KCl-induced release of dopamine from superfused striatal tissue slices obtained from rats 6 and 24 months of age.

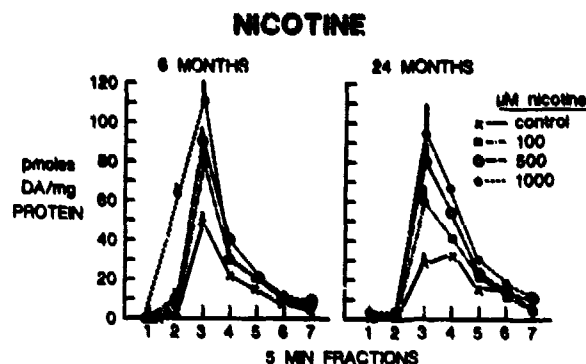


Fig. 7. Nicotine enhancement of 30 mM KCl-induced release of dopamine from superfused striatal tissue slices (6- and 24-month animals).

age groups (age by fraction by concentration — $F_{18,384} = 1.64$, $P < 0.05$). Further analyses showed that these differences were primarily the result of age-related differences in peak enhancement at the 100 μM concentration of nicotine ($t_{384} = 3.00$, $P < 0.02$). No significant age-differences were seen at higher concentrations.

Haloperidol-induced striatal DA antagonism

Direct DA autoreceptor inhibition with varying concentrations of haloperidol revealed only small age-related differences in the enhancement of KCl-induced release of DA (Fig. 7). While there was some overall lowering of striatal responsivity in the 24-month group (age by fraction by concentration — $F_{18,312} = 1.83$, $P < 0.05$), subsequent analyses indicated that each concentration of haloperidol was effective in enhancing the K^+ -evoked release of striatal DA (24-month within-age comparisons — 0 μM vs 100 μM, $t = 6.75$, $P < 0.001$; 100 μM vs 500 μM, $t = 1.76$, $P < 0.05$).

DISCUSSION

As stated in the Introduction, there are rather profound alterations in the various populations of striatal DA receptors, especially D_2 -receptors, that take place as a function of age. There are also indications from at least one recent study that the striatal DA autoreceptor is the D_2 -receptor⁶. Thus, it is interesting to note in this regard that, at least with respect to some of the independent variables assessed, the remaining D_2 -autoreceptors appear to show only mini-

mal age-related changes in function. Age-related differences in the K^+ -evoked release of DA from the striatal tissue slices were small in these experiments. These findings support those of earlier experiments³⁶, but appear to be somewhat different from those in which *in vivo* electrochemical analyses of striatal DA release was utilized to examine possible age-related differences in this parameter. A recent study by Rose et al.²⁹, in which this latter technique was employed, has indicated that two groups of 24-month-old animals showed no differences in K^+ -evoked release of striatal DA from that exhibited by 6-month animals. However, the third group of 24-month animals did show significantly lower K^+ -evoked release of DA when compared to the 6-month group. It is unclear why these within age-group differences exist since the experimental conditions were the same in all of the groups. The authors suggest there may have been slight differences in the rostrocaudal placement of the electrodes in these animals, since, as pointed out above, there are intraregional striatal variations in other indices of synaptic function. Therefore, some of the electrodes could have been placed into areas of the striatum that are relatively unaffected by aging.

Further evidence that DA autoreceptor function remains relatively unchanged during aging is indicated from the findings showing that there were only small between-group differences seen when the K^+ -evoked release of DA was enhanced with haloperidol. For example, in the presence of 30 mM KCl and 500 μ M haloperidol the DA release was enhanced about 37% in both the 6- and the 24-month groups.

One other source of evidence which indicates that there are no significant age-related declines in DA autoreceptor function can be found from the present results that indicate that there were only small differences in nicotine-enhanced K^+ -evoked release of DA in the striata obtained from the 6- and 24-month groups. Also important in this regard is that reciprocal inhibitory control between nicotinic heteroreceptors and DA autoreceptors is not lost with age. However, if RIC between muscarinic heteroreceptors is considered, there are strong indications of profound age-related changes. The results showed that there were selective and significant decreases in enhancement of KCl-induced release of DA from striatal slices by muscarinic agonists as function of age.

These decreases were highly dependent on the particular agonist that was used to enhance the release. Oxotremorine and pilocarpine were extremely effective in enhancing DA release and the release was generally greater when these partial agonists were applied than when a full agonist such as carbachol was placed in the medium. Age-related decrements in muscarinic control of the DA autoreceptor-mediated release of DA appeared as early as 12 months of age when carbachol was applied to the slices.

Previous studies (e.g. refs. 8, 9) have indicated that, based upon their ability to stimulate phosphoinositide turnover, these muscarinic agonists can be divided into two classes. Class A agonists, such as carbachol, maximally stimulate phosphatidate and inositol lipids from hippocampus and cortex, while partial agonists such as oxotremorine and pilocarpine only poorly stimulate phosphoinositide turnover. It is believed that class A agonists such as carbachol can alter the microenvironment of the muscarinic receptor (mAChR) allowing a change in the conformation/orientation of the receptor to a desensitized state, wherein the receptor is uncoupled from the effector mechanism²⁰. During aging, recent studies have suggested that, beginning with middle age, conformation/orientation changes take place in mAChR such that more mAChR are in a desensitized, decoupled state²⁰. If an agonist is applied which further desensitizes the mAChR, deficits in responding should be seen earlier in the lifespan than if an agent is utilized which does not induce decoupling of the receptor from the effector. Thus, age deficits are seen earlier with carbachol than with oxotremorine or pilocarpine.

Bethanecol, which was previously classed as a B (partial) agonist in hippocampus and cortex, behaves very much like an A agonist in the striatum and reductions in its efficacy are seen as early as 12 months of age. Preliminary results (not presented here) have indicated that the threshold concentration of bethanecol was 10 μ M in striata obtained from the 6-month group and 100 μ M in all other groups.

In summary then, the results of this and previous experiments indicate that although the age-dependent decreases in the concentrations of particular striatal DA subtypes do not appear to appreciably affect the functioning of those that remain, this loss combined with the decrease in concentration of stri-

tal mAChR and the increased heterogeneity of the mAChR can have a profound effect on the RIC that is normally exerted between these two systems. Since one can assume that proper striatal functioning depends upon the precise interplay between the ACh and DA systems, any age-related changes in their relationship could subsequently involve a myriad of other neurotransmitters and neuromodulators and be translated ultimately into motor behavioral deficits (i.e. decrements in balance, coordination, and sensory motor integration^{14,15,37,39}).

At present it is unclear what is responsible for the loss of striatal dopaminergic and cholinergic receptors in senescence. The relationship between receptor loss and neuronal loss is still controversial and investigations are being carried out in our laboratory as well as others. However, even if we were to suggest that the former is the result of the latter, this would not explain the selectivity of the age-related functional deficit in the mAChR, even though both DA and mAChR are lost with age. Moreover, it does not explain the mechanism of this functional loss.

One mechanism that is being explored to investigate this loss is that there may be accumulated membrane damage by free radicals. Some preliminary

data from our laboratory in support of this hypothesis have indicated that young animals irradiated with either high energy electrons or ⁵⁶Fe radiation (which are known to generate free radicals) also show reduced muscarinic enhancement of K⁺-evoked release of DA. These effects are coupled with motor behavioral deficits that resemble those seen in the senescent animal. Thus, some behavioral deficits seen with respect to aging and ionizing radiation may share a common mechanism, i.e., that of free radical-induced neuronal damage.

ACKNOWLEDGEMENTS

This work was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under work unit B4166. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources National Research Council.

REFERENCES

- 1 Cubeddu, L.X. and Hoffmann, I.S., Operational characteristics of the inhibitory feedback mechanism for regulation of dopamine release via presynaptic receptors, *J. Pharmacol. Exp. Ther.*, 223 (1982) 497-501.
- 2 Cubeddu, L.X. and Hoffmann, I.S., Frequency-dependent release of acetylcholine and dopamine from rabbit corpus striatum: its modulation by dopaminergic receptors, *J. Neurochem.*, 41 (1983) 94-101.
- 3 Cubeddu, L.S., Hoffmann, I.S. and James, M.K., Frequency-dependent effects of neuronal uptake inhibitors on the autoreceptor-mediated modulation of dopamine and acetylcholine release from the rabbit striatum, *J. Pharmacol. Exp. Ther.*, 226 (1983) 88-95.
- 4 Coupet, J., Rauh, C.E. and Joseph, J.A., Age-related loss of the capacity of muscarinic agonists to inhibit the activation of adenylate cyclase in the striatum, *Soc. Neurosci. Abstr.*, 11 (1985) 573.
- 5 Dubocovich, M.L. and Weiner, N., Modulation of the stimulation-evoked release of [³H]dopamine in the rabbit retina, *J. Pharmacol. Exp. Ther.*, 219 (1981) 701-707.
- 6 Dwoskin, L.P. and Zahniser, N.R., Robust modulation of [³H]dopamine release from rat striatal slices by D₂ dopamine receptors, *J. Pharmacol. Exp. Ther.*, 239 (1986) 442-454.
- 7 Farnetto, L.O. and Hamberger, B., Drug-induced changes in the release of [³H]monoamines from field-stimulated rat brain slices, *Acta Physiol. Scand.*, 84 (1971) 35-44.
- 8 Fisher, S.K., Klinger, P.D. and Agranoff, B.W., Muscarinic agonist binding and phospholipid turnover in brain, *J. Biol. Chem.*, 258 (1983) 7358-7363.
- 9 Fisher, S.K., Figueriedo, J.C. and Bartus, R.T., Differential stimulation of inositol phospholipid turnover in brain by analogs of oxotremorine, *J. Neurochem.*, 43F(1984) 1171-1179.
- 10 Gothert, M., Role of autoreceptors in the function of the peripheral and central nervous system, *Arzneim.-Forsch./Drug Res.*, 35 (1985) 1909-1916.
- 11 Henry, J.M., Filburn, C.R., Joseph, J.A. and Roth, G.S., Effect of aging on striatal dopamine receptor subtypes in Wistar rats, *Neurobiol. Aging*, 7 (1986) 357-361.
- 12 Hoffman, I.S. and Cubeddu, L.X., Rate and duration of stimulation determine presynaptic effects of haloperidol on dopaminergic neurons, *J. Neurochem.*, 39 (1982) 585-588.
- 13 James, M.K. and Cubeddu, L.X., Frequency-dependent muscarinic receptor modulation of acetylcholine and dopamine release from rabbit striatum, *J. Pharmacol. Exp. Ther.*, 229 (1984) 98-105.
- 14 Joseph, J.A., Bartus, R.T., Clody, D., Morgan, D., Finch, C., Beer, B. and Sesack, S., Psychomotor performance in the senescent rodent: reduction of deficits via striatal dopamine receptor up-regulation, *Neurobiol. Aging*, 4 (1983) 313-319.
- 15 Joseph, J.A., Berger, R.E., Engel, B.T. and Roth, G.S., Age-related changes in the nigrostriatum: a behavioral and

- biochemical analysis, *J. Gerontol.*, 33 (1978) 643-649.
- 16 Kamal, L.A., Arbilla, S. and Langer, S.Z., Presynaptic modulation of the release of dopamine from the rabbit caudate nucleus: differences between electrical stimulation, amphetamine, and tyramine, *J. Pharmacol. Exp. Ther.*, 216 (1981) 592-598.
 - 17 Keibbian, J.W. and Calne, D.B., Multiple receptors for dopamine, *Nature (Lond.)*, 277 (1979) 93-96.
 - 18 Kelly, M.J., An analysis of the effects of apomorphine and haloperidol on the release of [3 H]dopamine and [3 H]noradrenaline from rabbit brain slices, *Arch. Int. Pharmacodyn.*, 250 (1981) 18-29.
 - 19 Lehmann, J. and Langer, S.Z., Muscarinic receptors on dopamine terminals in the rat caudate nucleus: neuromodulation of [3 H]dopamine release in vitro by endogenous acetylcholine, *Brain Research*, 248 (1982) 61-69.
 - 20 Li, A.S., Loullis, C.C., Rotrosen, J., Cordasco, D.M., Critchett, D.J. and Joseph, J.A., Conformational changes in muscarinic receptors may produce diminished cholinergic neurotransmission and memory deficits in aged rats, *Neurobiol. Aging*, 6 (1985) 317-325.
 - 21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with Folin phenol reagent, *J. Biol. Chem.*, 193 (1951) 265-275.
 - 22 Morgan, D.G., Marcusson, J.O., Winblad, B. and Finch, C.E., Reciprocal changes in D-1 and D-2 dopamine binding sites in human caudate nucleus and putamen during normal aging [3 H]fluphenazine as a dopamine receptor ligand, *Soc. Neurosci. Abstr.*, 10 (1984) 445.
 - 23 Morin, A.M. and Wasterlain, C.G., Aging and rat brain muscarinic receptors as measured by quinuclidinyl benzilate binding, *Neurochem. Res.*, 5 (1980) 301-308.
 - 24 O'Boyle, K.M. and Waddington, J.L., Loss of rat striatal dopamine receptors with ageing is selective for the D₂ but not D₁ sites: association with increased non-specific binding of the D₁ ligand [3 H]piflutixol, *Eur. J. Pharmacol.*, 105 (1984) 171-174.
 - 25 Parker, E.M. and Cubeddu, L.X., Evidence for autoreceptor modulation of endogenous dopamine release from rabbit caudate nucleus in vitro, *J. Pharmacol. Exp. Ther.*, 232 (1985) 492-501.
 - 26 Plotsky, P.M., Wightman, P.M., Chey, W. and Adams, R.N., Liquid chromatographic analysis of endogenous catecholamine released from brain slices, *Science*, 197 (1977) 904-906.
 - 27 Raiteri, M., Marchi, M. and Maura, G., Presynaptic muscarinic receptors increase striatal dopamine release evoked by 'quasi-physiological' depolarization, *Eur. J. Pharmacol.*, 83 (1982) 127-129.
 - 28 Raiteri, M., Riccardo, L. and Marchi, M., Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain, *J. Pharmacol. Exp. Ther.*, 228 (1984) 209-215.
 - 29 Rose, G.M., Gerhardt, G.A., Conboy, G.L. and Hoffer, B.J., Age-related alterations in monoamine release from rat striatum: an in vitro electrochemical study, *Neurobiol. Aging*, 7 (1986) 77-83.
 - 30 Sakurai, Y., Takano, Y., Kohjimoto, Y., Honda, K. and Kamiya, H., Enhancement of [3 H]dopamine release and its [3 H]metabolites in rat striatum by nicotinic drugs, *Brain Research*, 242 (1982) 99-106.
 - 31 Severson, J.A. and Finch, C.E., Reduced dopaminergic binding during aging in the rodent striatum, *Brain Research*, 192 (1980) 147-162.
 - 32 Severson, J.A., Marcusson, J., Winblad, B. and Finch, C.E., Age-related changes in dopaminergic binding sites in human basal ganglia, *J. Neurochem.*, 39 (1982) 1623-1631.
 - 33 Starke, K., Reimann, W., Zumstein, A. and Heritting, G., Effect of dopamine receptor agonists and antagonists on release of dopamine from rabbit caudate nucleus in vitro, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 305 (1978) 27-36.
 - 34 Strong, R., Waymire, J.C., Samorajski, T. and Gottesfeld, Z., Regional analysis of neostriatal cholinergic and dopaminergic receptor binding and tyrosine hydroxylase activity as a function of aging, *Neurochem. Res.*, 9 (1984) 1641-1653.
 - 35 Thompson, J.M., Makino, C.L., Whitaker, J.R. and Joseph, J.A., Age-related decrease in apomorphine modulation of acetylcholine release from rat striatal slices, *Brain Research*, 299 (1984) 169-173.
 - 36 Thompson, J.M., Whitaker, J.R. and Joseph, J.A., [3 H]Dopamine uptake and release from striatal slices in young, mature, and senescent rats, *Brain Research*, 224 (1981) 436-440.
 - 37 Wallace, J.E., Krauter, E. and Campbell, B.A., Motor and reflexive behavior in the aging rat, *J. Gerontol.*, 35 (1980) 364-370.
 - 38 Watson, M., Yamamura, A.I. and Roeske, W.R., A unique regulatory profile and regional distribution of [3 H]pirenzepine binding in the rat provide evidence for distinct M₁ and M₂ muscarinic receptor subtypes, *Life Sci.*, 31 (1983) 2019-2023.
 - 39 Welford, A.T., Motor performance. In J.E. Birren and K.W. Schaie (Eds.), *Handbook of the Psychology of Aging*, Van Nostrand Reinhold, New York, 1977, pp. 450-497.
 - 40 Westfall, T.C., Besson, M.J., Giorgiueff, M.F. and Glowinski, J., The role of presynaptic receptors in the release and synthesis of [3 H]dopamine by slices of rat striatum, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 292 (1976) 279-287.
 - 41 Westfall, T.C., Grant, H. and Perry, H., Release of dopamine and 5-hydroxytryptamine from rat striatal slices following activation of nicotinic cholinergic receptors, *Gen. Pharmacol.*, 14 (1983) 321-325.
 - 42 Winer, B.J., *Statistical Principles in Experimental Design*, McGraw Hill, New York, 1971.